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Johnson et al (Arch. Virol. 140:623-634), 1995. Thanks!

Laurie Scheiner
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CM1 8E05
308-1122
8E12

ICP27 immediate early gene, glycoprotein K (gK) and DNA helicase homologues of infectious laryngotracheitis virus (gallid herpesvirus 1) SA-2 strain

M. A. Johnson, C. T. Prideaux, K. Kongsuwan*, S. G. Tyack, and M. Sheppard**

CSIRO Division of Animal Health, Animal Health Research Laboratory,
Parkville, Victoria, Australia

Accepted December 15, 1994

Summary. A 4.8 kilobase segment located at the left-terminal in the unique long (U_L) region of infectious laryngotracheitis virus (ILTV) SA-2 strain contained three open reading frames (ORFs). The first of 421 amino acids (aa) was located at map units 0.065 to 0.07, and its predicted 48 kiloDaltons (kDa) protein product has significant homology to the immediate early regulatory protein ICP27 (UL54) of herpes simplex virus type-1 (HSV-1), to varicella-zoster virus (VZV) ORF4 and to equine herpesvirus 1 (EHV-1) ORF5. The zinc finger conserved in the C-terminal of the proteins from HSV-1, VZV and EHV-1, is poorly conserved in ILTV homologue. The second ORF of 336 aa, located at map units 0.075 to 0.08, has a predicted molecular weight (MW) of 38 kDa with significant homology to glycoprotein K (gK) of HSV-1 (UL53), ORF5 of VZV and ORF6 of EHV-1. ILTV gK has features characteristic of a membrane-bound glycoprotein. The 3' region of a third ORF was located at map units 0.08 to 0.095. Translation of the sequence revealed significant homology to the 3'-region of the DNA helicase-primase complex protein (UL52) of HSV-1, ORF6 of VZV and ORF 7 of EHV-1. Northern blot analyses were used to characterize the ILTV ICP27, gK and DNA helicase mRNAs. The data revealed that ILTV ICP27 is an immediate early gene that encodes a 1.6 kb mRNA, ILTV gK encodes a late transcript of 1.8 kb, while ILTV DNA helicase encodes a late transcript of 3.7 kb.

Introduction

Infectious laryngotracheitis virus (ILTV) or Gallid herpesvirus 1 is a member of the family *Herpesviridae*, subfamily *Alphaherpesvirinae* [27, 28]. ILTV can

*Current addresses: CSIRO Division of Tropical Animal Production, Long Pocket Laboratory, Indooroopilly, Queensland, Australia; ** SmithKline Beecham, Lincoln, Nebraska, U.S.A.

cause a severe respiratory disease in chickens primarily affecting the mucous membranes of the larynx and trachea [4]. In the most severe cases, death occurs within 2–3 days after the first signs of illness and can result in mortality rates as high as 70%, although they are usually between 10–40% [4, 11]. The disease is controlled in Australia by the use of a commercial vaccine of a small plaque variant SA-2 [35].

ILTV has previously been shown to have a class D genome of 155 kilobase pairs (kb) characterized by a unique long (U_L) region of 120 kb and a unique short (U_S) region of 17 kb bounded by internal (IR) and terminal repeats (TR) of 9 kb [16, 22]. Random DNA sequencing allowed the identification of 21 ILTV genes by sequence homology to other herpesviruses [9]. Of these genes, 20 had significant sequence homology to genes of varicella-zoster virus (VZV) and 19 to genes of herpes simplex virus (HSV), both alphaherpesviruses. Sequence data of the thymidine kinase (TK) gene and upstream genes provided evidence for an evolutionary relationship between ILTV and other alphaherpesviruses [10].

A complete restriction map for the SA-2 strain is now available [16], and has allowed the positioning of the glycoprotein B gene [18] and other genes such as thymidine kinase, DNA binding protein and the major capsid protein (unpubl. res.). Each of these genes was found in collinear positions with those of the HSV homologues. Similarly, other genes including the immediate early ICP4 homologue [17], protein kinase [20] and a glycoprotein in the gG family [19] were located in collinear positions with the HSV prototype. In this report, we present the sequence data for three genes located between 0.065–0.095 map units at the left hand end of the U_L region, interpret its genetic content and discuss ILTV relatedness to other herpesviruses.

Materials and methods

Growth of ILTV

Primary chicken kidney (CK) cells were obtained from 2 to 4 week old specific pathogen free (SPF) white leghorns and grown in monolayers at 37 °C in minimal Basal Medium Eagle (BME) with Earle's salts, 2 mM glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, 20 U/ml polymyxin, 10 µg/ml neomycin, 2.5 µg/ml fungizone and 10% foetal calf serum. Strain SA-2 of ILTV was supplied by Arthur Websters Pty Ltd. (Castle Hill, Australia) and is the vaccine strain used in Australia. A working stock was obtained by infecting a 10 cm petri dish of CK cells with the initial stock and incubating the cells at 39 °C until they showed >90% cytopathic effect (cpe). Since ILTV is largely a cell-associated virus, the cells were scraped, pelleted, the media removed and the cells resuspended in SPGA (0.2 M sucrose, 0.01 M potassium phosphate, 0.05 M glutamic acid pH 7.0). The titre of the working stock was 5×10^7 PFU/ml. Virus stocks were frozen (liquid nitrogen) and thawed three times prior to infection to release ILTV from cells.

Preparation of ILTV DNA

CK cells in 100 10 cm petri dishes were infected at 1 PFU/cell and incubated at 39 °C until 80–90% cpe. The cell pellet was resuspended in TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) and extracted twice with NP40 at a final concentration of 1% on ice for 15 min. SDS was

added to a final concentration of 0.5% to the supernatants and was extracted with 1:1 (vol/vol) phenol:chloroform, followed by ethanol precipitation and final resuspension in TE.

Sequencing of DNA

Plasmids used in sequencing were cloned as described previously [16]. Clones containing *Eco*RII (pJJ089) and *Bgl*II fragments pJJ274 and pJJ312 were prepared for double stranded sequencing using the dideoxynucleotide chain termination method [32]. After initial alkali denaturation of the DNA template [12], DNA was labelled using [³⁵S]dATP (Amersham) and the *Taq* polymerase sequencing kit (Promega) and separated on 6% polyacrylamide, 8 M urea buffer. Gels were dried onto glass plates and exposed to Fuji RX film for 1 to 3 days. Oligonucleotides for use in DNA sequencing were made on an Applied Biosystems 392 DNA-RNA Synthesizer, N-butanol precipitated directly from ammonia solution [34] and used without further purification. Both strands were sequenced. Sequences were analyzed using the DNASIS and PROSIS packages (Pharmacia). Alignments were made using CLUSTAL V package [13].

Isolation of mRNA

Monolayers of primary CK cells were prepared in 50 mm petri dishes (1×10^6 cells) and infected with ILTV at a multiplicity of 10 PFU per cell. Cells were treated 2 h prior to infection with cycloheximide (200 µg/ml), then infected for 4 h in the presence of cycloheximide to enrich for immediate early mRNA [14]. To enrich for early mRNA, phosphoacetic acid (PAA) was added (50 µg/ml) to the cell culture medium 6 h post-infection (p.i.) and the mRNA isolated 10 h p.i. No metabolic inhibitors were used to isolate late mRNA with infected cells being harvested 16 h p.i. Poly(A) mRNA from mock- or ILTV-infected CK cells was prepared using the PolyA Tract mRNA isolation system (Promega).

Northern blot analysis

Poly(A) RNA (0.5 µg) was fractionated on 1% agarose gels containing 2.2 M formaldehyde, 50% formamide as described by Sambrook et al. [33]. After electrophoresis, RNA was transferred onto Hybond-N+ nylon filters (Amersham, U.K.) by the method of Southern [36]. RNA molecular weight markers were from Promega. Oligonucleotides were used to PCR the coding regions of the genes, which were purified from agarose gels, labelled with [α -³²P] dATP using random hexamers and the Klenow fragment of DNA polymerase (Promega) and hybridized to RNA on filters. DNA-RNA hybridizations were performed at 42 °C for 16 h in 50% formamide, 5 × SSC, 20 mM sodium phosphate (pH 6.5) 1 × Denhardt's solution and 0.1% SDS. Hybridization filters were washed at 42 °C in 0.1 × SSC and 0.1% SDS prior to autoradiography.

Results and discussion

4811 nucleotides of DNA sequence have been lodged with GenBank, accession no L34065. The segment sequenced was located at the left-terminal of ILTV, map units 0.065 to 0.095, and included *Eco*RI fragments I, g' and a portion of F (Fig. 1).

Gene arrangement and function

Open reading frames (ORFs) were located by searching for ATG initiation codons and candidate polyadenylation sites (AATAAA) near the termini. Three

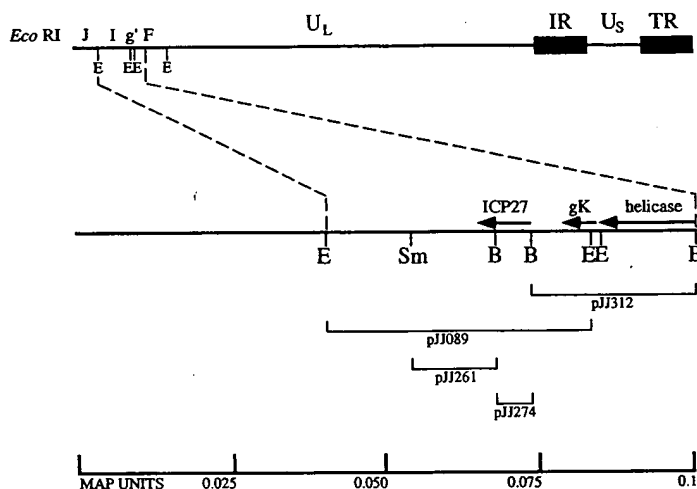


Fig. 1. Map of the infectious laryngotracheitis virus genome. Map unit positions are shown. U_L Unique long region; U_S unique short region; TR terminal inverted repeat of the unique short region; IR internal inverted repeat of the unique short region. The clones used to sequence the 4811 nt segment reported in this publication are enlarged below the ILTV map. Map locations and direction of ILTV ICP27, gK and DNA helicase homologues are shown

ORFs were found, the direction of transcription was from right to left on the complementary strand (Fig. 1). The encoded proteins were then compared with translated sequences of HSV-1, VZV, EHV-1, Epstein Barr virus (EBV), channel catfish virus (CCV) and some proteins of Marek's disease virus (MDV), herpesvirus of turkeys (HVT), and pseudorabies virus (PRV), obtained from the GenBank Data library.

ILTV ICP27 homologue

The ORF located at nt 3356 to 4618 was found to be an homologue of HSV-1 UL54 (ICP27; IE63; IE3) [24], VZV ORF4 [7] and EHV-1 UL3 [39] or ORF5 [37] (Fig. 2). The predicted ILTV ICP27 protein is 421 aa and 48 kDa. A potential initiation of transcription motif (TATA) was found 477 bp upstream from the initiation codon and a polyadenylation signal was located 106 nt downstream of the stop codon. GC rich regions were also found upstream of the start codon. In HSV-1, ICP27 is a 63 kDa alpha phosphoprotein that localizes to the nuclei of infected cells [1]. ICP27 is an essential gene in the regulation of early and late gene expression and is required for virus replication [23, 30]. The hydropathy plot of ILTV ICP27 showed a similar pattern to that of HSV-1 ICP27 [24, 31]. The amino half of the protein is highly hydrophilic (rich in arginine and serine), whilst the carboxyl terminal is relatively hydrophobic. The carboxyl half of HSV-1 ICP27 has been shown to be required for activation and repressor functions in the regulation of early and late gene expression [30]. It has also been shown that a potential zinc finger (metal binding domain) is found in the carboxyl terminal region of HSV-1 ICP27 [24, 38], HSV-1 IE110

[25] and EHV-1 UL3 [39] (Fig. 3). Alignment of the last 100 aa of ILTV ICP27 showed that possibly the first and last cysteine residues of the proposed consensus zinc finger [2,3] are conserved, while the second cysteine and histidine residues have undergone the transitions Cys → Ala and His → Lys. This arrangement in ILTV ICP27 is different from that found in HSV-1, EHV-1 and VZV. However, studies on frame shift mutants showed that the zinc finger motif could be varied and still bind zinc in vitro [38]. Alignment of the ILTV ICP27 protein with ICP27 of HSV-1, ORF4 of VZV and UL3 of EHV-1 showed at the conserved amino acid level homologies of 20, 18 and 18%, respectively, and at the conservative substitution level, 60, 60.5 and 62% respectively. There were also a number of residues conserved across all ICP27 homologues in the carboxyl-terminal. These residues may be important for structure or for activator or repressor functions. Northern blot analysis (Fig. 4A) using clone pJJ274 as a probe showed a 1.6 kb immediate early (IE) transcript of the predicted size and larger transcripts. The 5.4 kb transcript was the result of a previous study on ILTV ICP4. However, we do not know what the other 4.8 kb transcript is, and may be the result of residual protein synthesis in the presence of cycloheximide. Several other transcripts were found early (E) and late (L) in infection (Fig. 4A), and no 1.6 kb transcript was detected. The detection of these transcripts is most likely due to the overlap of sequences in the probe (pJJ261) with 5' sequence of the next gene downstream from ILTV ICP27. The number of transcripts indicates that several of these genes share 3' co-terminal mRNAs. As no 5' or 3' transcription studies have been done, we do not know where these other transcripts map, or if any products are associated with these transcripts and, therefore, will require a more detailed investigation.

ILTV glycoprotein K (gK) homologue

The ORF located at nt 1537 to 2550 was found to be a homologue of HSV-1 UL53 (gK; cell fusion protein) (DebRoy et al. [8]; McGeoch et al. [24]), VZV ORF5 (Davison and Scott [7]) and EHV-1 ORF6 (Telford et al. [37]) or UL4 (Zhao et al. [39]). No initiation of transcription motif (TATA) was located 5' to the initiation codon. A polyadenylation signal was located 10 nt 3' of the stop codon. A GT rich region was also found 24 nt 3' of the stop codon. These GT regions following polyadenylation signals are associated with RNA cleavage [5]. A second polyadenylation site was located in the 806 bp region between ICP27 and gK. A similar feature was found for the region between HSV-1 UL53 and UL54 (DebRoy et al. [8]; Pogue-Guile and Spear [26]). However, this differed from the region between UL3 and UL4 of EHV-1 (Zhao et al. [39]) and the region between ORF4 and ORF5 of VZV (Davison and Scott [7]) both of which lack a polyadenylation signal and comprise transcripts that shared a 3' co-terminal (Zhao et al. [39]; Inchauspe et al. [15]). Potential glycosylation sites are located at aa 66 to 68 (NSS) and aa 98 to 100 (NET) (Fig. 5). Trans-membrane domains were located between aa 124 to 145, aa 219 to 235, aa 251 to 273 and aa 306 to 322, and a potential signal sequence was found between aa 1 and 30

DNA Helicase ->

R S D T S I R D S C L L M R S D A P L A D Q A M L P V I D L P W T E
 AGATCTGACACCAGCATACGCGACTCCTGCTTACTAATGCGCAGTGATGCCCCCTCGCAGATCAGGCCATGTTGCCAGTAATAGACTTGCCTTGGACGG 100
 C N E S K P H G E L V R K I K E G Q S C S G L S Q M Y L N R N E V
 AGTGCAATGAATCGAAACCGCATGGGGAAGTAGTTGCAAAAAATAAATTCGGGCAGTCTTGACGCGGTCTGTCACAAATGTACCTGAATAGGAATGAAGT 200
 L N E S L A I Q S L I L D I D I P L K L D H G P I T M L T L H K A
 CCTTAACGAATCTCTAGCAATTCAATCCCTGATACTGGATATCGACATACCCCTCAAGCTAGATCACGGCCCCATAACGATGTTGACCTTACATAAAGCG 300
 M R A V R V A L I Q L I A L L F P E T S I S H D T Y P V Y F Y K S H
 ATGCGGGCGGTGCGCGTGGCATTGATCCAATTAATAGCCTTATTGTTCCGGAGACATCAATCAGTCATGACACCTATCCCGTGTACTTTTACAAAAGTC 400
 C S S P E S H D A Q Y S S V D I D F L E N T C S D D I E A E E N M
 ACTGCTCGTCCCCGGAGCCATGACGCACAATATTCTCAGTGGATATTGATTTTTTGGAGAATACATGTTCTGATGACATAGAAGCAGAAGAGACAT 500
 Y V C D S G W V E E M S W C D E H A S E H G A I V S V Q D L Q D A
 GTAGCTCTGGGATAGCGGCTGGGTAGAAAGATGTGATGGTGTGATGAGCATGCGAGTGAACATGGAGCCATAGTCTCTGTTCAAGACTTACAGGATGCG 600
 L N V F R D R A N S T L C K C K E K L G F R V C V P I P S P Y A L F
 TTAATGTATTTCCGGATCGTGCAATTAACCTTATGCAAAATGCAAGAAAAGCTCGGGTTCAGGGTCTGCGTCCCAATCCCATCCCATATGCTTTGT 700
 G L E T V K T I A N V A Q H V I L L Q E E F I E C L D D V I R D Y
 TTGGTCTAGAGACAGTAAAACTATCGGGAACGTAGCTCAGCAGTAAATTTACTGCAGGAAGAATTCAATGAATGTTTAGACGATGTTATCAGAGACTA 800
 D F I D T G I Y S P G R S L R L P F F A K V S E S G F M S G R L L
 TGACTTTATTGACACTGGAATCTACTCGCCAGGACGAAGCCTCGGCTACCTTTCTTGGCAAGGTTCTGAATCCGGATTCAATGCTGGGAGACTCCTG 900
 P F I I F P P D C A D K V A F A A A H K D P N N F H F H A F R P D N
 CCGTTTATAATTTTCCCCCTGACTGTGCGAGATAAAGTAGCTTTCGGGCGAGCGCACAAAGACCCCTAATAATTTCCATTTTCATGCTTCCGTCCAGACA 1000
 P T P N I I V T R I A C P P D V I G G V S K P S S N L P Q T A V S
 ATCCCAACCCAAATATTATGCTCACTCGAATCGCGTGTCTCCTGACGTTATAGGGGGAGTTTCAAAACCCCTCAGCAACTTGCCCCAACGGCTGTGTC 1100
 L S L V E A F S R V N L A P C E D R T G A G V D T I D G V F V L T
 TCTTAGCCTCGTGGAGCATTTCAGTCGTGCAATTTAGCACCGTGCGAAGACAGGACAGGAGCGGGTGGATACGATCGATGGAGTCTTTGTGCTCAGG 1200
 T Y V L P A I T N Y I K E H F P S L A H E Y S D I S F G D V R V L K
 ACATATGTTCTGCGGGCCATAACAAATTACATAAAGAGCATTTTCCATCGTTGGCGCAGCAATACAGCGACATTAGTTTGGAGACGTGCGGGTGCTCA 1300
 T R I T A S L L R N R R G C G G R A T F T C L K H S H R S A A A Q
 AAACGCGCATTACCGCATCACTATTACGAATCGTCGAGGGTGCGGAGGGGCGCAACTTTTACCTGCCTTAAACATTGCGATCGAAGTGCCGCTGCCCA 1400
 T V I T S V A V A I N S Q G N P Y A A F Q N Q M L C D
 GACCGTCATTACTTCTGTAGCGGTGCAATTAACCTCACAGGGCAATCCCTACGCGGCATTTCAGAACCAATGCTTTGGGACTAAGTGCGGAGGCAACAC 1500
 G K -> M L R P E C L K W A V I L T G T I H I V F L
 GCTGCAAACTCAGTTTACAGTGCCTGTGACCAAGAATGCTGCGACCAAGATGTCTAAATGGGAGTAAATTTAACCGGAACGATACATATAGTCTTCT 1600
 V W Y V C S K I S T D S K D D C L Y V L A N I K W L L P G Q Q E F
 TGGTATGGTATGTATGCTCTAAAATTTCCACTGACTCAAAGGACGACTGCTTGACGTACTCGCAAAATATTAAATGGCTCCTGCCGGACAGCAAGAATT 1700
 N P V H Q P P A T E N S S L V Y V L T K Y T Q R L N N Y E N H Q T
 CAATCCTGTTTACCACACCCCGCTACATTTAATCTTCACTAGTTTATGCTCTACAAATATACACAGCGTCTAAACAACTACGAGAACCACCAGAGG 1800
 R C V K G A Y F E N E T V L I S R L I P L A K E Q Y S S W K W Q T V
 AGATGTGTTAAAGGAGCTTATTTTGAAGATGAGACTGTACTCATTCTCGTCTGATCCCACTGGCCFAGAACAGTATTGCTCATGGAAGTGGCAGACTG 1900
 S L H M V F P D Q S C I S T V I V H M L L A D P C Q R R M F G S V
 TCTCTTTACATATGTTTTCAGATCAGAGTTGCATTCCACGGTTATTGTACATATGTTACTTGCTGACCCGTGCCAGAGGCGAATGTTCCGGCTCTGT 2000
 C R E N A L R L D A Y H L N Y W T A F T S R L I L R V P Y T K M Q
 CTGCCGCGAGAACGATTGCGATTGGATGCAATCATCTAACTACTGGACAGCGTTTACTTCGAGGCTGATATTACGGGTGCCATACACAAAGATGCAA 2100
 R F L R E F E H V R D C K S L N Y V A D P L G F C I C N P G V L V L
 CGGTTTTTGGGGAATTTGAACATGTCCGAGATTGCAAAAGCTTGAAGTACGTAGCAGACCCCTCTAGGCTTTTGCATCTGTAATCCAGGGGTCTTAGTAC 2200
 K T L E I G L Y L A S L I M S T M T L R I C Y D P C A Y I L H E H
 TGAAAACACTCGAGATCGGTTTATATTAGCATCGCTTATTATGTCACCATGACATTGCGGATTGCTATGATCCGTGTGCATATATTTACATGAACA 2300
 V K I S A W V Y V I V S A V L E L L S L M G Y T T P A K T K V S A
 CGTAAAAATTAGTGCTTGGGTATATGTAATGTCTCAGCGGTTCTAGAACTCTTACTAGTATGGGTACACGACTCCGGCAAGACTAAAGTCTCCGCA 2400
 S K P P S I L T S C L A N I A S S L V L R A L C V A A I A S I V I I
 TCGAAACCTCCAGTATCTTGACTTCATGCCTTGCCAATATTGCTTCGAGCTTAGTCTTGGTGCATTTGCGTGGCTGCGATTGCGAGCATTTGAATAA 2500
 A F K Y E Q K I Q N K L F G P
 TTGCATTTAAATACGAACAGAAGATACAAAACAAATTTGTCGGGCTTGAACGGCAATAAATGTTAAACACGTTGTGCGGTGTTGTGCTGAATTTGG 2600

polyA

G/T rich region

TCCATTTGAAGACAGCCCTAGTTCTAACGGCTGAGTATCATTGTTTGAAGGAGTAACATCTGGCGGTGAGAATGTACAAAGTATACTCGTGGGTGATT 2700
TAGGGCGTGTCTACTAAGCGGATGTTTTCGGGTTTAGACGGCATGCCGACTACATTACGGGATTAAGTTACATGTCCGATCCGCAGAAGTTCTCGCAGA 2800
TTATTATGTCTGCGTGAATATTTGCGACCACTACTGGATCGCAATTGTAGTTTGTGGGTTCCAGTGTATGTGTTATGACGTATACTGGGAGTGGCCAG 2900
ACCATTCAAAAGCCAAGTGGATTAGCTCGCAACAGCGTGCCAGATTACTGTGAGCTTACCTTTTACGACCTGCTCCGAGCTTGACACCTGTTCTGAGTC 3000
CGTAGAAGTCTCAACCGTTGTGTATGCGGTTTCATTTTTTTTACGCTGCACGCGGTACATCAACCCACTGACTGCGATGGAGACCGGAGAAATCTGGAAG 3100
AAATAAGATAGCGTGAGACAGTTTACAGAGCATCTTCAATACAAGTACTCATAGCGAAGCGAGATCAGCAACGGCCCGGACAAAAGATCGCCCTGGATGG 3200
polyA
CCGTTACGCTGAGGTGAGAATCCCGCCACGAAGCACATTGTATTCGCCGAGATCTCATTCTACAGAAAGTAGCTCCAGATCCCGGTTTCATCTGGGCGC 3300
ICP27 -> M A R P R S R Y E N Q S G M S
GGTACGTCCAAACGAGTCCGTGATTCAAGTGACAGGGGGCGCGTTCGCTGTTTGAATGGCGAGGCTCGTTCCTCGTTACGAGAACCAGTCAGGTATGAGC 3400
d/C rich region
V R S S I R R V S S P R N K F M R G R A H I Q V R R G I P P R P R R
GTACGGTCTTCCATAAGGCGGGTCAGTAGCCCTCGAAATAAGTTTATGAGAGGCGGTGCGCATATCCAAGTCCGAAGAGGCATCCCCCTAGACCCAGGC 3500
R A G T P E K R Y R A P I F T V S L K H S R R S W E R N R D E L R
GCCGTGACGTACACAGAAAAGCGATATAGGGCGCTATCTTTACTGTTTCGTTGAAGCATTGCGCGAGGTCTTGGGAAAGAAATCGGGATGAACCTCG 3600
R P I W R D E V R C P T S S R T E T K E L R N V T P A Q Y F E K A
AAGACGATTGGAGAGACTTGTCCGATGCCAACGTATCACGGACCGAAACGAAGGAGTTGCGAAACGTAACACCGGCCCAATATTTGAAAAGGCC 3700
A T A F G G L G K C I T E E L R L E N Q K C L L D M V N R A V D D D
GCAACTGCATTGCGGGTCTCGGAAAGTGCATTACTGAAGAGTTAAGATTAGAAAATCAGAAATGTCTTTTAGACATGGTAAACCGGGCAGTGGATGATG 3800
D C D D I D R D R G I C F P A F L S S G S S D L A A D A A F T S W
ATGATTGTGATGATATCGATCGTGATAGGAATCTGCTTTCCAGCATTTTGTCTTCGGGATCATCTGACCTTGCAGCCGATGCTGCATTCACTTCGTG 3900
K Q F C G R A A S L K G R W T S R P D I A R L A K I S R A V Y L A
GAAGCAGTTTGTGGGCGCGCAGCTTCACTGAAAGGCGCTGGACATCGCGTCCGGATATAGCCAGGTTGGCAAAAATTTACAGAGCTGTATATTGGCG 4000
N C S F E E L L F A C D E T L V W M L W H Q F E D E R I Y P H D P I
AACTGCTCATTTGAAGAGCTACTTTTTCGATGCGATGAGACACTTGTATGGATGCTTGGCATCAGTTCGAAGATGAAAGGATTACCCCCACGATCTA 4100
F S N I Y C A C Q S L A M H L G P I L P C Y L S S I G S Q L R D T
TCTTCTTAACATCTACTGCGCATGTCAATCTCTAGCCATGCATCTGGGCCAATCCTGCCGTGTTATCTCTAGCATTGGCAGTCAACTAAGAGATAC 4200
T R S Q E L S L S S A K C P L T L L L T F F D R F S R I V Y P R S
CACTAGATCTCAGGAGCTATCACTGAGTAGCGCAAAATGTCTTTAACTTTATTACTGACCTTCTTCGACCGGTTCTCAAGAATTGTGTATCCGCGATCA 4300
E A I V M N H K A I D P A R T L W D M Y Y P G T C S K K I P L V L R
GAGGCCATAGTCATGAATCATAAAGCAATAGACCCGGCCAGAACATTGTGGGACATGTACTATCCTGGGACCTGTTCTAAAAAATCCCACTCGTTCTGC 4400
S T Q M C A A K R N A E W F V R S S K P Q Y T V G K F S L A T C L
GCAGCAGCAAAATGTGTGCGCAAGAGGAATGCAGAGTGGTTTGTGCGCAGCTCTAAGCCGAGTATACAGTAGGGAAATTTCTCTTGGCACTTGTTT 4500
T V L Y T Y R H M A V L Y W N W C H P T F L K I A I S V T G Q Q M
GACAGTTCTATACACATATCGACACATGGCGGTATTGTATTGGAATGGTGCCATCTACGTTTCTGAAGATTGCCATCTCTGTGACTGGTCAGCAGATG 4600
A P R A Q
GCGCCGCGAGCTCAATGATGTAAGCGTTCACTGTTGCAATCCGGCCTTGTAATAACACTGTAGTATTAGCAGGTTGAAAATTTTGAATGTTCCCGGA 4700
CTCTCGAAAATTAACGGAGGTATAATAAAGAACACAATACAACGGACACCGACAACGTGGAGCATTTTATTAGTCTGCAAACTCTTGGTTGATGAACG 4800
polyA
TGTTGCCCTG 4811

Fig. 2. Nucleotide sequence of the 4811 nt segment containing ILTV ICP27, gK and DNA helicase homologues. *Cis*-acting regulatory sequences such as TATA boxes, polyadenylation signals (poly A) and GT rich regions are indicated. The deduced amino acid sequence is shown above the nucleotide sequence. Only the 3' region of the DNA helicase gene is shown

(Fig. 5). The overall conserved amino acid identity with HSV-1 gK, EHV-1 UL4 and VZV ORF5 is 26.7, 25 and 27%, respectively, and at the conservative substitution level, 64.8, 63 and 62%, respectively. Northern blot analysis revealed a faint early transcript of 1.8 kb and a predominant late transcript of 1.8 kb (Fig. 4B). Other faint transcripts of higher molecular weight were also seen and are most likely 3' co-terminal transcripts, as no polyadenylation signal is found between ILTV gK and the gene 5'.

		a.a.
ILT_ICP27	MAR-----PRSRYENQSGM-----	14
HSV_ICP27	MATDIDMLIDLGLDLSDDLDEDPPEPAESRRDDLESSSGECSSSDEDMEDPHGEDGPE	60
VZV_ORF4	MASA-SIPTDPDVSTICEDF---MNLDPDEPSDDFALEVTD--WANDEAIGSTPGEDSTT	54
EHV1_orf5	MA-----LSSVSSCEPMEDEMSIMGSDTEDNFTGGDT--CAEATRGLVNKSAFVPTQ	50
	**	
ILT_ICP27	----SVRSSIRRV-----SSPRNKF-----	31
HSV_ICP27	PILDAARPAVRPSRPEDPGVPSTQTPRPTERQGPNDPQPA-PHSVWSRLGARRPSCSPEQ	119
VZV_ORF4	S-----RTVYVERTADTAYNPRYSKRRHGR-----RESYHHNRPKTLVVVLPSDN	99
EHV1_orf5	TV--GTVSALRNVGDPKSVVVSFSASPQRAQPSNPKSERPAFGHGRNRNRRPFRRNNW	108
 *	
ILT_ICP27	----RGRAHIQVRRGIPPRRRRAGTPEKRYRAPIFTVSLKHSRR-----	72
HSV_ICP27	HGGKVARLQPPPTKAQPARGGRRRRGRGRGGPGAADGLSDPRRRAPRTNRNPGGPRPG	179
VZV_ORF4	HHGGR---DVETGYARIERGHRRSSRSYNTQSS-----RKHRDRSLSNRRRRPTTPPA	149
EHV1_orf5	KQQQRGWKEPEPENV-PARQSA-GSWPKRS-----SLPVHMLGQR-----GGDSSS	153
 *	
ILT_ICP27	-SWERNRDELRRPIWRDFVRCPTSSRTETKELRNVT-----AQYFEKAA	116
HSV_ICP27	AGWTDGPGAPHGEAWRGSEQDPDPGGQRTGRVQAPPPLMTLAIAPPPADPRAPAPERKA	239
VZV_ORF4	MTTGERNDQTHDES YRL--RFSKRDARRERIRKEYDIPV-----	186
EHV1_orf5	ADSGHGGAGP-SDRWRFKTRTQSA-RVHRNRRRGN-----ANHGSNTPGR--	197
 *	
ILT_ICP27	TAFGGLGKCITEELRLLENQKCLLDVMNRAVDDD-----DCDDIDRDRGICFP AFLSS	168
HSV_ICP27	PAADTIDATRLVLRISISERAAVDRISESFGRSAQVMHDPFGGQPPFAANS-PWAPVLAG	298
VZV_ORF4	---DRITGRAIEVVSTAGASVTIDSVRH-LDETIEKLVVRYAT-----IQEGDSWAS--	234
EHV1_orf5	SAGDRLNAAAASSIADVCRRTVSSRIGEMFHGARETLTPVKNGGFRAENSSPWAPVLGF	257
 *	
ILT_ICP27	GSSDLAADAFTSWKQFCGRAASLKGRWTSRPDIARLAKISRAVYLANCSFEELLFACDE	228
HSV_ICP27	QGGPFDAETRRVSWETLVAHGPSLYRTFAGNPRAASTAKAMRDCVLRQENFIEALASADE	358
VZV_ORF4	-GGCFPGIKQNTSWPELMLYGHLYRTFESYKMSRIARALRERVIRGESLIEALESAD	292
EHV1_orf5	GSDQFNPEARITWDTLVEHGVNLYKLFVRSASHAAEAARS LRDAVMRGENLLEALASADE	317
 *	
ILT_ICP27	TLVWMLWHQFEDERIYPHPDPIFSNIYACQSLAMHLGPILPCYLSSIGSQLRDTTRSQ--	286
HSV_ICP27	TLAWCKMCIHHNLPLRPQDPIIGTTAAVLNDNLATRLRPFLQCYLKAR---GLCGLDEL	414
VZV_ORF4	LLTWIKMLAAKNLPITYNNPIVATSKSLLENLKLKLGPFVRCLLLNRDNDLGSRTLPELL	352
EHV1_orf5	TLWCKMIVTKNLPMTTRDPIISSVALDNLRLKLEPFMRCYLSSS---GSPTLAELC	373
	* * *	
ILT_ICP27	-ELSLSSAKCPLTLLLTFFDRFSRIVYPRSEAIVMNHKAIDPARTLWDMYYPGTCSKKIP	345
HSV_ICP27	SRRRLADIKDIASFVVFILARLANRVERGVAEIDYATLGVGVEKM-HFYLPACMAGLI	473
VZV_ORF4	RQORFSDITCITTYMFVMIARIANIVVRGSKFVEYDDISCNV-QVL-QEYTPGSCLAGVL	490
EHV1_orf5	DHQRSLDVACVPTFMFVMLARIARAVGSAETVSRDALGPD-GRVL-ADYVPGACLAGTL	431
 *	
ILT_ICP27	LYLRSTQM-CAAKRNAEFVRRSSKPQYTVGKFSLATCLTVLYTYRHMAVLYWNWCHPTFL	403
HSV_ICP27	EILDTHROECSS-RVCELTA-SHIVAPPYVHGKYFYC-----NSLF-	512
VZV_ORF4	EALITHQRECGR-VECTLSTWAGHLS DARPYGKYFKC-----STFN	451
EHV1_orf5	EAIDAHKRRCKA-DTCSLVS-AYTLVPVYLHGKYFYC-----NQIF-	470
 *	
ILT_ICP27	KIAISVTGQQMAPRAQ	429
HSV_ICP27	-----	512
VZV_ORF4	C-----	452
EHV1_orf5	-----	470

Fig. 3. Alignment of the amino acid sequences of ILTV ICP27, HSV-1 ICP27 (McGeoch et al. [24]), VZV ORF4 (Davison and Scott [7]) and EHV-1 UL3 (Zhao et al. [39]). Numbers at the right indicate the number of amino acids from the N-terminus. Symbols: * conserved amino acids across all for sequences; . conserved or conservative substitutions across two or more sequences

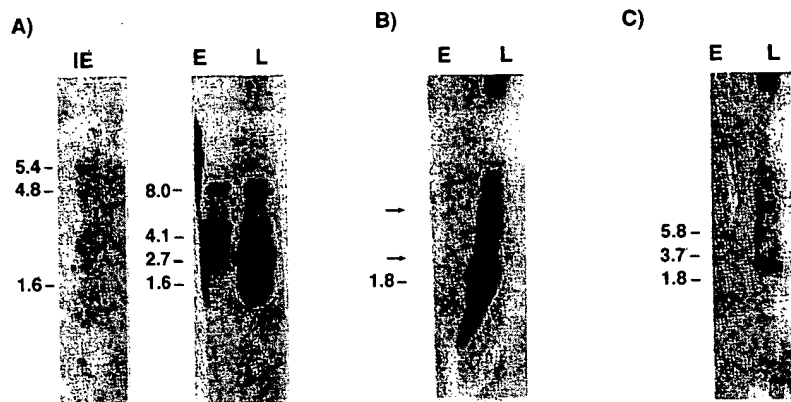


Fig. 4. A Northern blot analysis of the ILTV ICP27 transcript. Immediate early (*IE*) mRNA was isolated 4 h post-infection (see Materials and methods). Clone pJJ274 (Fig. 1) was labelled and hybridized as described in Materials and methods. The size of the transcripts are indicated. This filter was previously probed with sequences coding for the ILTV immediate early gene ICP4 (5.4 kb transcript). Clone pJJ261 which contains 3' sequence of ILTV ICP27 and sequence of putative ORFs downstream was used to probe containing early (*E*) and late (*L*) mRNA. B Northern blot analysis of the ILTV gK transcript. Oligonucleotides were used to PCR the open reading frame. This fragment was labelled and hybridized to filters containing early (*E*) and late (*L*) mRNA. The size of the transcript is indicated. C Northern blot analysis of the ILTV DNA helicase transcript. Oligonucleotides were used to PCR the 3' region of open reading frame. This fragment was labelled and hybridized to filters containing early (*E*) and late (*L*) mRNA. The size of the transcripts is shown

DNA helicase homologue

The sequence located at nt 1 to 1485 was found to be homologous to the 3' region of the HSV-1 UL52 (DNA helicase-primase complex) (McGeogh et al. [24], Crute et al. [6]), VZV ORF 6 (Davison and Scott [7]) and EHV-1 ORF 7 (Telford et al. [37]) (Fig. 2). The DNA helicase induced upon HSV-1 infection consists of three sub-units encoded by UL52, UL5 and UL8. This enzyme is essential for replication and is also associated with DNA primase activity. Northern blot analysis showed several late transcripts of 5.8, 3.7 and 1.8 kb (Fig. 4C). The transcript of 3.7 kb is the predicted mRNA of ILTV helicase, based on the size of the coding region of other alphaherpesvirus helicase homologues (VZV, 3249 nt; EHV-1, 3243 nt). The detection of multiple late transcripts of similar size for both probings of ILTV gK and helicase indicated 3' co-terminal mRNAs

We have now sequenced a number of genes of ILTV and located their positions on genomic maps of the ILTV SA-2 vaccine strain (Johnson et al. [16]). Based on amino acid sequence homologies it appears that these genes are conserved in relative position between ILTV and other alphaherpesviruses. The arrangement of genes and structure of the left-terminus of ILTV is similar to that found for other herpesviruses. The presence of these highly conserved genes

	signal	a.a.	
ILT_gK	ML---RPECLKWAVILTGTIHIVFLVWVCSKISTDSKDDCLYVLANIKWLLPGQQEFN	56	
HSV_UL53	MLAV-RSLQHLSTVVLITA--YGLVLVWY--TVFGASPLQHRRIYAVRPT-----GTNN	48	
VZV_ORF5	MQALGIKTEHFIIMCLLSG--HAVFTLWY--TA-RVKFEHECEVYATTVI-----NGGP	48	
EHV1_ORF6	ML-LGGRTAYLSVLGLITA--YAAFTIWIY--TL-TAQLHNPCVYATVSI-----DSKD	47	
	*. ** *		
	+++	+++	
ILT_gK	PVHQPPATFNSSLVYVL-----TKYTQRLNNYE----NHQTRCVKGAYFENETVLISRL	106	
HSV_UL53	DTALVWMKMNQTLTLFLGAPTHPPN---GGWRNHAHICYANLIAGRVPFQVPPDAMNRRI	105	
VZV_ORF5	VV---WGSYNNSLIYVTFVNHST--FLDGLSGDYDSRENLLSGDTMVKTAISTPLHDKI	103	
EHV1_ORF6	GIAAKWEVYNSTIVY--AYPENGAKRFS DGLSGFDYVCRENWVNESKLDVLKNMKELHDKV	105	
	*.		
	124	145	
ILT_gK	-IPLAKEQYSSWKWQTVSLHMFVFPDQSCISTVIVHMLLADPCQRRMFGSVCRENAL-RLD	164	
HSV_UL53	MNVHEAVNCLETW-YTRVRLVV-----VGWFLYLAFVALHQRRCMFGVVS PAHKMVAPA	159	
VZV_ORF5	RIVLGTNRCHAYFW-CVQLKMIF-----FAWVYGYMYLQFRRIRRMFGPFRSSCELISPT	157	
EHV1_ORF6	RIVVGTNRNCRAYLW-SVQLQMIT-----GAWLIYIAFLCLRQERRLLGPFRNQNEFLSPT	160	
	*. . . . *		
	219		
ILT_gK	AYHLNYWTAFTSRILIRVPYTKMQRFLREFEHVRDCKSLNYVADPLGFCICNPGVLVLKT	224	
HSV_UL53	TYLLNYAGRIVSSVFLQYPYTKITRLCELSVQRQNLVQLFETDPVTFLYHRPAIGVIVG	219	
VZV_ORF5	SYSLNYVTRVISNILLGYPTKRLARLLCDVSMRRDGMKSVFNADPISFLYMHKGVTLML	217	
EHV1_ORF6	GYTFNYATYTLATTVLKTHYTKFALLCEASLRRVALSRTFKRDPIGFLCEHSAALALIG	220	
	*. . . . *		
	235	251	273
ILT_gK	LEIGLYLASLIMSTMTRICYDPCAYILHEHVKISAWVYVIVSAVLELLSLMGYTTPAKT	284	
HSV_UL53	CELMLRFVAVGLIVGTAFISRGACAITYPFLFTITTWCFVSTIGLTLYCILRRGPAPKN	279	
VZV_ORF5	LEVIAHISGCI VLLTLGVAYTPCALLYPTYIRILAWVVVCTLAIVELISYVRPKPTKDN	277	
EHV1_ORF6	LEVGTHTFVARLLVVGTVTLVHTPCSQIYPIYLKLSWGFVVAVTIVEIVAIIEYKPPKGT	280	
	*. . . . *		
	306	322	
ILT_gK	KVSASKPPSIL-----TSLANIASSLVLRALCVAASIVIAFKYEQKIQNKLF--	335	
HSV_UL53	ADKAAAPGRSGKLSGVCGRCCSIIILSGIAVRLCYIAVAVGVVLVALHYEQEIQRRLFDV-	338	
VZV_ORF5	HLNHINTG---GIRGICTTCCATVMSGIAIKCFYIVIFAIAVVFIMHYEQRVQVSLFGES	334	
EHV1_ORF6	SSANPPTPATHGVKGLCTSCCSTVLANLCGKLVYLLLVIGAVSILLHYEQRIQIGLLGES	340	
	*. . . . *		
ILT_gK	-----P	336	
HSV_UL53	-----	338	
VZV_ORF5	ENSQKH	340	
EHV1_ORF6	FSS---	343	

Fig. 5. Alignment of the predicted amino acid sequences of ILTV gK, HSV-1 UL53 (DebRoy et al. [8]), ORF5 of VZV (Davison and Scott [7]) and UL4 of EHV-1 (Zhao et al. [39]). Numbers at the right indicate the number of amino acids from the N-terminal. Symbols: * conserved amino acids across all four sequences; . conserved or conservative substitutions across two or more sequences. The potential N-terminal signal sequence and four transmembrane domains for ILTV gK are indicated by lines above the ILTV gK sequence. Potential N-linked glycosylation sites are indicated by + + +

in ILTV provides further evidence of the evolution of the alphaherpesviruses from a common progenitor. The levels of conserved identity between the ILTV genes in this study and homologues of other alphaherpesviruses was low, with high levels of conservative substitutions, indicating that considerable divergence has occurred. The reporting of these essential genes provides a framework for future studies on the basic molecular biology of ILTV.

Acknowledgements

We thank Julie Harris and Som Spiess for the preparation of the tissue culture. This work was supported by Arthur Websters Pty Ltd, Castle Hill, Australia. The sequences have been lodged with the Genbank Database, Accession no. L34065.

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Authors' address: Dr. M. A. Johnson, CSIRO Division of Animal Health, Animal Health Research Laboratory, Private Bag 1, Parkville, Victoria 3052, Australia

Received October 20, 1994